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Acaulospora spinosissima, a new arbuscular mycorrhizal fungus from the Southern Guinea Savanna in Benin

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A new arbuscular mycorrhizal fungus was isolated from the Southern Guinea savanna in Benin, which represents a tree-rich savanna in the transition between the tropical atlantic rainforests and grass-rich savannas in sub-Saharan West Africa. The fungus was propagated in bait cultures and monosporic single species cultures, and is here described as *Acaulospora spinosissima*. It forms spores similar to those of *Acaulospora spinosa*, but in *A. spinosissima* the outer wall is thinner and the surface ornamentation is finer. Sequences obtained from the ITS and the partial 28S of the ribosomal gene revealed that the two species are phylogenetically not closely related. The new fungus was recovered from natural savanna at two locations and from

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one field site under yam cultivation in the first year after tree clearance. It was not detected in agricultural field sites cultivated for more than one year.

Keywords: Glomeromycota, taxonomy, arbuscular mycorrhiza, savannas, biodiversity.

Spore surface ornamentation is a common feature for species of arbuscular mycorrhizal (AM) fungi in the family Acaulosporaceae (Tab. 1). Currently there are 15 species described with a pitted spore surface (e.g. Trappe 1977, Oehl *et al.* 2011, Furrazola *et al.* 2013), two species having pits beneath spiny projections (Gerdemann & Trappe 1974, Rothwell & Trappe 1979), one species with pits on top of projections (Sieverding & Toro 1987), and eight species with spiny to pustulate projections without any other ornamentation on the same spore wall (Walker & Trappe 1981, Janos & Trappe 1982, Palenzuela *et al.* 2013, Cai *et al.* 2013).). There are also 20 species with smooth to rugulate spore surfaces (e.g. Gerdemann & Trappe 1974, Schenck *et al.* 1984, Morton 1986, Błaszkowski 1988 a, 1995, 2012, Oehl *et al.* 2012).

In recent years, remarkable progress has been achieved regarding the identification and description of *Acaulospora* species with pitted spore surfaces (e.g. Błaszkowski 1988 b, 1989, 2012, Oehl *et al.* 2006, 2012), also due to advances made in molecular phylogenetic analyses. However, clear morphological identification of species with smooth spore surfaces or projections on the spore surface has remained difficult because of the lack of clearly differentiating features (Palenzuela *et al.* 2014).

In the current study we describe a new AM fungus that has projections on the spore surface, which superficially resemble those of *A. spinosa* and *A. tuberculata*. Molecular analyses on the ribosomal gene clearly separated the fungus from both these species phylogenetically, positioning it near to *A. herrerae* and *A. kentinensis*.

Materials and methods

Study sites

Study sites comprised 27 natural, fallow, and cultivated agro-ecosystems, located within the Sudan (SU), Northern Guinea (NG), and Southern Guinea (SG) savanna ecological zones of Benin, sub-Saharan West Africa as described in Tchabi *et al.* (2008). The SG savanna has two wet and two dry seasons per year, while the NG and SU savannas are monomodal. The selected sites were either natural savannas or cultivated yam (*Dioscorea* spp.) fields established in the first year after (forest) savanna clearance, mixed cropping systems, groundnut (*Arachis hypogaea*) or intensively managed cotton (*Gossypium hirsutum*) fields. Sites located in long-term fallows (≥7 years old) were also included to compare species occurring in undisturbed sub-Saharan savannas with those present in restored fallows and under varying levels of cropping intensification and soil disturbance, represented by crop cultivation along the rotation cycle, where yam is the first crop after

Tab. 1. *Acaulosporaceae* species described worldwide.

Acaulospora species	Year of publication
Species with smooth spore surface	
Acaulospora capsicula Błaszk.	1990
A. colossica P. A. Schultz *et al.*	1999
A. delicata C. Walker *et al.*	1986
A. endographis G. T. Goto	2013
A. entreriana M. S. Velásquez & Cabello	2008
A. gedanensis Błaszk.	1988
A. koskei Błaszk.	1995
A. laevis Gerd. & Trappe	1974
A. longula Spain & N. C. Schenck	1984
A. mellea Spain & N. C. Schenck	1984
A. morrowiae Spain & N. C. Schenck	1984
A. polonica Błaszk.	1988
A. splendida Sieverd. & S. Toro	1988
A. sporocarpia M. S. Berch	1985
A. thomii Błaszk.	1988
A. viridis Palenz. *et al.*	2014
A. walkeri Kramad. & Hedger	1990
Kuklospora colombiana Spain & N. C. Schenck	1984
Species with roughened spore surface	
Acaulospora dilatata J. B. Morton	1986
A. rugosa J. B. Morton	1986
Species with projections on spore surface	
A. colliculosa Kaonongbua *et al.*	2010
A. pustulata Palenz. *et al.*	2013
A. soloidea Vaingankar & B. F. Rodrigues	2011
A. spinosa C. Walker & Trappe	1982
A. spinosissima Oehl *et al.*	Herein
A. tortuosa Palenz. *et al.*	2013
A. tuberculata Janos & Trappe	1982
Kuklospora spinosa B. P. Cai *et al.*	2013
Species with pitted spore surface	
A. alpina Oehl *et al.*	2006
A. cavernata Błaszk.	1989
A. excavata Ingleby & C. Walker	1994
A. foveata Trappe & Janos	1982
A. herrerae Furrazola *et al.*	2013
A. kentinensis (C. G. Wu & Y. S. Liu) Kaonongbua *et al.*	2010
A. lacunosa J. B. Morton	1986
A. minuta Oehl *et al.*	2011
A. nivalis Oehl *et al.*	2012
A. paulinae Błaszk.	1988
A. punctata Oehl *et al.*	2011
A. rehmii Sieverd. & S. Toro	1987
A. scrobiculata Trappe	1977
A. sieverdingii Oehl *et al.*	2011
A. taiwania H. T. Hu	1988
Species with projections on pitted spore surface	
A. bireticulata F. M. Rothwell & Trappe	1979
A. elegans Trappe & Gerd.	1974
Species with pits on top of projections of spore surface	
A. denticulata Sieverd. & S. Toro	1987

land clearance and cotton is furthest along the cycle. The tree-rich forest savanna vegetation of the Guinea savannas consists of trees, shrubs and grasses with tree and shrub prominence decreasing from south to north (e.g. Adjakidje 1984, Adjanohoun 1989, Tchabi *et al.* 2008). The soils are predominantly ferruginous Ferralsols.

Soil sampling and culturing of AM fungi

Soils were sampled as described in Tchabi *et al.* (2009 a), towards the end of the 2004 wet season in September/October and during the subsequent dry season in February 2005. Soil pH, organic carbon, and available phosphorus were determined using standard methods (Tchabi *et al.* 2008, 2009 b). The spore material used during the study originated directly from field samples. Extensive attempts were made to propagate the AM fungal species present in the field samples through 'bait' cultures using various hosts (*Brachiaria humidicola*, *Stylosanthes guianensis, Sorghum bicolor*, *Dioscorea cayenensis*, *D. rotundata*). Several bait culture systems were also established (Tchabi *et al.* 2008, 2009 a) inoculating 5–10 % field soils to autoclaved substrate (Terragreen: Quartz sand mixture; 3:1 [wt/wt]). The AM fungal communities were cultivated for eight months and the host plants periodically analyzed for mycorrhizal infection and AM fungal spore formation. Spores of the new fungus were detected in bait cultures from three sites originating in the SG Savanna (Tab. 2).

Monosporic cultures on *Hieracium pilosella* were established from spores isolated from bait culture samples that had been air-dried and stored for three months before inoculation (Tchabi *et al.* 2009 a). AM symbiosis establishment and new AM fungal spore formation succeeded in six monosporic isolates, with three isolates originating from natural savanna forest site 'ns3' in the village Tobe-Koko of the county Banté, two isolates originating from soil from a yam production field site adjacent to 'ns3' 'yf3', and one isolate originating from soils from natural forest savanna site 'ns2' in the county Savé. Isolates were recovered from soil samples taken in the dry season (February 2005).

Morphological analyses

Spores were extracted from the bait cultures and the six monosporic pure cultures by wet sieving and sucrose centrifugation (Sieverding 1991) before being mounted in PVLG, PVLG + Melzer's reagent (Brundrett *et al.* 1994), and water (Spain 1990). Terminology used in the species description follows Oehl *et al.* (2011) and Palenzuela *et al.* (2011) for species with spore formation laterally on the neck of sporiferous saccules. Photographs in Figs. 1–8 were taken using a Leika DFC 295 digital camera mounted on a Leica DM750 compound microscope using Leica Application Suite Version V 4.1.0 software. Specimens mounted in PVLG and the mixture of PVLG + Melzer's reagent were deposited at Z+ZT (mycological herbarium at ETH Zurich,

Switzerland) and URM (mycological herbarium of the Federal University of Pernambuco in Recife, Brazil) herbaria.

Molecular analyses

Crude extracts were obtained by crushing five surface-sterilized spores with a sterile disposable micropestle in 40 µL milli-Q water, as described by Ferrol *et al.* (2004). The spores derived from one monosporic pure culture (isolate FO438) originating from spores of the type location at the savanna forest site 'ns3'. Spores were surface-sterilized with chloramine T (2 %) and streptomycin (0.02 %) (Mosse 1962), and washed in sterile water thereafter. PCR's were performed in an automated thermal cycler (Gene Amp PCR System 2400, Perkin-Elmer, Foster City, CA, USA) with a pureTaq Ready-To-Go PCR Bead (Amersham Biosciences Europe GmbH, Germany) following manufacturer's instructions with 0.4 µM concentration of each primer. A two-step PCR was conducted to amplify the ribosomal fragment consisting of partial SSU, ITS1, 5.8S, ITS2 and partial LSU rDNA using the primers SSUmAf/LSUmAr and SSUmCf/LSUmBr consecutively according to Krüger *et al.* (2009). PCR products from the second round of amplifications were separated electrophoretically on 1.2 % agarose gels, stained with Gel Red™ (Biotium Inc., Hayward, CA, USA) and viewed by UV illumination. The band of the expected size was excised with a scalpel and isolated from the gel with the QIAEX II Gel Extraction kit (QIAGEN, USA) following the manufacturer's protocol, cloned into the pCR2.1 vector (Invitrogen, Carlsbad, CA, USA), and transformed into One Shot® TOP10 chemically competent *Escherichia coli* (Invitrogen, Carlsbad, CA, USA). Recombinant colonies were selected by blue/white screening and the presence of inserts detected by PCR amplification directly from white colonies with GoTaq® Green Master Mix (Promega) using universal forward and reverse M13 primers. After isolation from transformed cells, plasmids were sequenced on both strands with M13R/T7 primers using the BigDye Terminator kit 3.1v (Applied Biosystems). The products were analyzed on an automated DNA sequencer (Perkin-Elmer ABI Prism 373). Sequence data were compared to gene libraries (EMBL and GenBank) using BLAST programs (Altschul *et al.* 1990). The new sequences were deposited in the EMBL database under the accession numbers HG422732- HG422734.

Phylogenetic analyses

The phylogeny was reconstructed by independent analyses of the ITS region and partial LSU rDNA. The AM fungal sequences obtained were aligned with other related glomeromycotan sequences from GenBank in ClustalX (Larkin *et al.* 2007) and the mismatches in the alignment were corrected manually using BioEdit (Hall 1999). *Claroideoglomus etunicatum* W. N. Becker & Gerd. was included as an outgroup. Prior to phylogenetic analysis, the model of nucleotide substitution was estimated using Topali 2.5

(Milne *et al.* 2004). Bayesian (two runs over 2×10^6 generations, with a sample frequency of 200 and a burnin value of 25 %) and maximum likelihood (1000 bootstrap) analyses were performed in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) and PhyML (Guindon & Gascuel 2003), respectively, launched from Topali 2.5, using the GTR + G model. Maximum parsimony analyses were performed with PAUP*4b10 (Swofford 2003), using a heuristic search with 1000 bootstrap replications and the following parameters: stepwise addition = random, branch swapping = TBR.

Results

Taxonomy

Acaulospora spinosissima Oehl, Palenz., I. C. Sánchez, Tchabi, Hount. & G. A. Silva, **sp. nov.** – Figs. 1–8.
MycoBank no.: MB 804884

Diagnosis. – Sporae singulae lateraliter formatae ad sacculum terminalem, flavae ad fusco-flavae, globosae vel subglobosae, 120–187 × 116–180 µm in diametro. Tunica exterior spinulis regularibus, 0,5–1,1 µm altis et 0,4–0,8 µm latis. Differt ab *A. spinosa* habens tunica exteriore et ornamentatione tenuioribus. Holotypus Z+ZT (ZT Myc 52169).

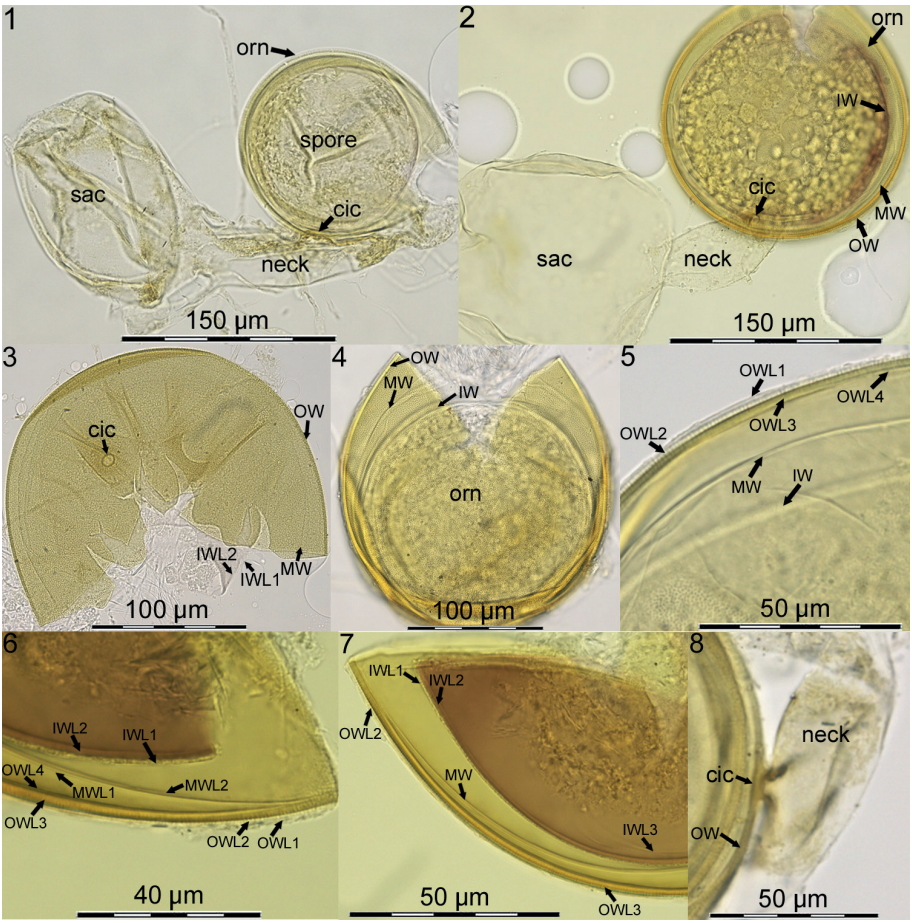
Etymology. – Latin, *spinosissima*, referring to the subtle spiny ornamentation on the outer spore surface.

Holotypus. – Cultivated in monosporic cultures at the University of Basel, Institute of Botany, on *Hieracium pilosella*, collection number 39–3901, deposited at Z + ZT (common mycological herbarium of the University and ETH of Zurich, Switzerland, ZT Myc 52168). Field soil was originally sampled by Atti Tchabi and Louis Ezin Lawouin from a forest savanna in Benin, Southern Guinea savanna, in the county Savè (07° 57. 217' N; 002° 26. 935' E; 141 m a.s.l.). Isotypes deposited at Z+ZT (ZT Myc 52169) and GDA-GDAC (herbarium of the University of Granada, Spain). Paratypes were isolated from bait cultures and monosporic cultures inoculated at University of Basel, Institute of Botany, with spores or soil samples originating from a yam production site and an adjacent forest savanna in the county Banté, respectively (08°19.661' N; 001°51.340' E, 250 m a.s.l. and 08° 19.730' N; 001° 51. 332' E; 250 m a.s.l.) deposited at Z+ZT (ZT Myc 52170 and 52171), GDA-GDAC and URM.

Characters. – Sporiferous saccules are hyaline and formed singly at the end of mycelial hyphae. The saccule termini are globose to subglobose (120–188 × 115–185 µm), with 1–2 wall layers that are in total 1.8–2.8 µm thick (Figs. 1–2). The saccule usually collapses after the spore has formed and usually is detached from mature spores in soils. Saccule neck at terminus is 25–38 µm wide, often inflating between terminus and spore base, where it is 20–38 µm wide tapering to 5.5–10 µm within 50–100 µm from the spore base.

Spores (Figs. 1–4) form laterally, in distance of 35–80 µm, on the neck of sporiferous saccules. They are globose to subglobose, 120–187 × 116–180 µm in diameter, rarely elliptical to oblong. They are light yellow when young, becoming bright yellow to brownish-yellow with age, and have three walls.

The outer spore wall consists of four layers (OWL1–OWL4) and is in total 2.5–4.6 µm thick (Figs. 4–7). The outer layer (OWL1) is hyaline to subhya-



Figs. 1–8. *Acaulospora spinosissima* (holotype and isotypes): **1–2.** Spore formed laterally on the neck of a sporiferous saccule with three walls (OW, MW, IW); cicatrix (cic) at spore base and spiny ornamentation (orn) at spore surface visible. **3–4.** Crushed spores with three relatively thin spore walls. **5.** Outer wall with four layers (OWL1-OWL4). OWL2 with subtle spiny projections. **6–7.** Spore wall structure in Melzer's reagent. MW with two layers (MWL1-MWL2); IW with three layers (IWL1-IWL3) of which the IWL1 is 'beaded', IWL2 staining purple in Melzer's reagent, and IWL3 thin and difficult to see when adhering to IWL2. **8.** Cicatrix on OW at spore base.

line, 0.5–0.8 µm thick and evanescent. The second layer (OWL2) is subhyaline, densely crowded with short spiny projections that are 0.5–1.1 µm high and 0.4–0.8 µm wide at base. The third layer (OWL3) is light yellow becoming dark yellow to brownish yellow with age, finely laminated, 1.0–2.7 µm thick. The inner layer of the outer wall (OWL4) is concolorous with OWL3, about 0.5 µm thin and often difficult to observe.

The middle wall is hyaline, bi-layered and 0.7–1.8 µm thin in total. Both layers (MWL1 and MWL2) are semi-flexible, tightly adherent to each other and often appear as a single wall layer (Figs. 4–7).

The inner wall is hyaline (Figs. 4–7), with two to three layers (IWL1–IWL3) that are 1.6–3.7 µm thick in total. The IWL1 is 0.8–1.2 µm thick with a 'beaded', granular structure, which is rarely seen in lactic acid based mountants. IWL2 is 0.8–1.5 µm thick and regularly stains dark pink to purple in Melzer's reagent. IWL3 is very thin and usually very difficult to detect due to the close adherence to IWL2.

The cicatrix (Figs. 1, 2, 8) remains visible at the spore base after detachment of the connecting hypha, and is 8.5–13 µm wide. The pore is closed by some of the inner lamina of OWL2 and by OWL3.

M o l e c u l a r a n a l y s e s . – The phylogenetic analysis from ITS rDNA sequences confirms the new fungus in a clearly separate clade (Figs. 9–10). From partial sequences of the LSU rDNA, *A. herrerae* and *A. kentinensis* are the species occurring most closely to *A. spinosissima* (95 and 94 % of identity, respectively). For the ITS region, the closest species related to *A. spinosissima* is *A. kentinensis* with 86 % identity. The intraspecific variation between the different clones of *A. spinosissima* was around 1–2 % for the LSU rDNA and 1–4 % for the ITS sequences. No environmental ITS or partial LSU rDNA sequences deposited in the GenBank correspond to *A. spinosissima* in the BLASTn analysis.

D i s t r i b u t i o n . – The new fungus has so far been recovered from soil at three sites in Benin, two from sub-Saharan forest savannas and one from a cultivated yam production site in its first season following clearance of the natural forest savanna at 140–250 m a.s.l. Mean annual air temperatures are about 27 °C in these sites. Topsoil pH was 6.2–7.2 at the three sites. Organic carbon contents were 20–24 and 38–44 g kg^{-1} in the two forest savanna sites and 6.5–7.5 g kg^{-1} in the yam production site. Available P contents were also higher in the natural forest savanna sites than in the yam production site (27–38 mg kg^{-1} and 3.9–13.1 mg kg^{-1} respectively, Tab. 2).

The new fungus jointly occurred in field samples with several other AM fungal species. These included several *Glomus* (e.g. *G. macrocarpum* and *G. clavisporum*), *Funneliformis* (e.g. *F. mosseae*), *Claroideoglomus* (e.g. *C. etuni-*

Fig. 9. Phylogenetic tree of the Acaulosporaceae obtained by analysis from ITS1, 5.8S rDNA and ITS2 sequences of different *Acaulospora* spp. Sequences are labeled with their database accession numbers. Support values (from top) are from maximum parsimony (MP), maximum likelihood (ML) and Bayesian analyses, respectively. Sequences obtained in this study are in boldface. Only support values of at least 50 % are shown. Thick branches in grey represent clades with more than 90 % of support in all analyses. The tree was rooted with *Claroideoglomus etunicatum*. Scale bar indicates the number of base substitutions per site. (Consistency Index = 0.53; Retention Index = 0.86).


Claroideoglomus etunicatum AJ239125
C. etunicatum AF004683
Kuklospora colombiana FR750063
K. colombiana AJ239117
Acaulospora entreriana FR750172
A. entreriana FR750169
A. entreriana FR750171
A. colossica AF133764
A. colossica AF133770
A. colossica AF133772
A. laevis AJ242499
A. laevis FM876786
A. laevis FM876780
A. lacunosa AJ891112
A. lacunosa AJ981111
A. lacunosa AJ891113
A. tortuosa HF567933
A. tortuosa HF567937
A. tortuosa HF567934
A. alpina AJ891101
A. alpina AJ891103
A. alpina AJ891106
A. pustulata HF567941
A. pustulata HF567940
A. pustulata HF567939
Acaulospora sp. FN825906
Acaulospora sp. FN825904
Acaulospora sp. FN825902
Acaulospora sp. FN825907
A. minuta FR821674
A. minuta FR869690
A. minuta FR821672
A. spinosa FR750151
A. spinosa FR750155
A. spinosa FR750153
A. scrobiculata FR692349
A. scrobiculata FR692351
A. scrobiculata FR692350
A. spinosissima HG422732
A. spinosissima HG422734
A. spinosissima HG422733
A. kentinensis FN547522
A. kentinensis FN547520
A. kentinensis FM876828
A. delicata JF439203
A. morrowiae AJ242500
A. mellea AJ239116
A. morrowiae AM905249
A. delicata JF439093
A. mellea JF439090
A. mellea JF439092
A. cavernata FM876789
A. cavernata FR692348
A. cavernata FR692347
A. punctata FR846383
A. punctata FR846384
A. punctata FR846385
A. nivalis HE603643
A. nivalis HE603644
A. nivalis HE603642
A. denticulata AJ239115
A. paulinae AJ891114
A. paulinae AJ891116
A. paulinae AJ891115
A. sieverdingii AM076376
A. sieverdingii FM876793
A. sieverdingii AM076378
63
87
0.86

71
0.90
86
72

78
55
1.00
62
79
0.99
0.1

Tab. 2. Geographic position and selected chemical soil parameters at sampling sites in Benin where *Acaulospora spinosissima* was found.

Sampling sites	Geographic position	Elevation (m a.s.l.)	pH (H_2O)		Organic C g kg^{-1}		Available P (Na-acetate) mg kg^{-1}		Available P (citrate) mg kg^{-1}	
			w.s.	d.s.	w.s.	d.s.	w.s.	d.s.	w.s.	d.s.
Natural Savanna 1 'ns2' in Savè	07° 57.217' N; 002° 26.935' E	141	7.2	7.2	44.1	37.7	27.3	27.0	37.6	35.8
Natural Savanna 2 'ns3' in Banté	08° 19.661' N; 001° 51.340' E	250	6.5	6.9	20.3	23.8	28.8	21.8	34.9	30.6
Yam field 'yf3' in Banté	08° 19.730' N; 001° 51.332' E	250	6.2	6.3	6.4	7.5	6.5	3.9	8.7	13.1

Natural forest savannas (ns2-3) were undisturbed for at least 25–30 years, and the yam field (yf3) established during the first year following forest clearance. Site abbreviations follow Tchabi *et al.* (2009 a).

catum), *Acaulospora* (e.g. *A. scrobiculata*, *A. minuta* and *A. spinosa*) and *Racocetra* (e.g. *R. beninensis* and *R. tropicana*) species (e.g. Tchabi *et al.* 2009 b, Goto *et al.* 2011, Oehl *et al.* 2011) among others.

Discussion

The new AM fungus *Acaulospora spinosissima* can easily be distinguished from all other species in the Acaulosporaceae through combined morphological and molecular analyses. Phylogenetically, it forms a separate clade on both ribosomal gene regions investigated and is distant to the morphologically most similar species, *A. spinosa* and *A. tuberculata*, which form spiny or tuberculate ornamentations on the spore surface. Of these species, *A. spinosissima* forms the thinnest walled spores, which is true for all three spore walls, and especially for the outer wall and the wall layer of the spiny ornamentations, called tuberculate in *A. tuberculata*. It is likely, however, that due to the morphological similarities between *A. spinosissima* and *A. spinosa* differentiation may be difficult using field collected spores.

Two additional *Acaulospora* species possess spiny ornamentation on the spore surface, *A. elegans* and *A. bireticulata*. These two species, however, have pitted ornamentations below the spiny layer, resulting in species with double ornamentations on the spore surface.

Fig. 10. Phylogenetic tree of the Acaulosporaceae obtained by analysis from partial LSU rDNA sequences of different *Acaulospora* spp. Sequences are labeled with their database accession numbers. Support values (from top) are from maximum parsimony (MP), maximum likelihood (ML) and Bayesian analyses, respectively. Sequences obtained in this study are in boldface. Only support values of at least 50 % are shown. Thick branches in grey represent clades with more than 90 % of support in all analyses. The tree was rooted with *Claroideoglomus etunicatum*. Scale bar indicates the number of base substitutions per site. (Consistency Index = 0.53; Retention Index = 0.86).


Claroideoglomus etunicatum AF145749
C. etunicatum AJ623310
Kuklospora colombiana FJ461804
Kuklospora sp. FJ461805
Kuklospora sp. FJ461810
Acaulospora koskei FJ461793
A. entreriana FR750173
A. entreriana FR750171
A. laevis FM876787
A. laevis FM876780
A. laevis FM876786
A. pustulata HF567938
A. pustulata HF567940
A. pustulata HF567939
Acaulospora sp. FN825902
Acaulospora sp. FN825904
Acaulospora sp. FN825906
A. tortuosa HF567935
A. tortuosa HF567936
A. tortuosa HF567932
A. alpina FR681926
A. alpina FR681927
A. alpina FR681929
A. colliculosa GU326343
A. colliculosa GU326344
A. colliculosa GU326345
A. lacunosa FJ461800
A. lacunosa AJ510230
A. foveata FJ461801
A. herrerae JX135572
A. herrerae JX135571
A. herrerae JX135573
A. spinosissima HG422732
A. spinosissima HG422734
A. spinosissima HG422733
A. kentinensis FJ461808
A. kentinensis FN547520
A. kentinensis FN547522
A. spinosa FR750155
A. spinosa FR750151
A. spinosa FJ461798
A. tuberculata FJ461799
A. scrobiculata FR692350
A. scrobiculata FR692349
A. scrobiculata FR692351
A. minuta FR821672
A. minuta FR821673
A. minuta FR821674
Amorrowiae FJ461795
A. dilatata FJ461792
A. longula AM040291
A. longula AM040292
A. delicata JF439203
A. delicata FJ461791
A. mellea JF439091
A. mellea JF439092
A. cavernata FM876789
A. cavernata FR692348
A. cavernata FR692347
A. punctata FR846383
A. punctata FR846384
A. punctata FR846385
A. nivalis HE603643
A. nivalis HE603642
A. nivalis HE603644
A. paulinae AY639328
A. paulinae AY639327
A. sieverdingii FM876793
A. sieverdingii FJ461796
A. sieverdingii FM876792
0.1

Spiny projections on the spore surface are a well-known feature in Glomeromycota, for example as described for *F. monosporus* (Gerdemann & Trappe 1974), *G. spinosum* (Hu 2002) and *G. spinuliferum* (Oehl *et al.* 2003), *Scutellospora spinosissima* (Walker *et al.* 1998), *A. spinosa* (Walker & Trappe 1981) and *A. tuberculata* (Janos & Trappe 1982) and *Kuklospora spinosa* (Cai *et al.* 2013). In addition *Pacispora dominikii* has been described with spiny to tuberculate projections on the spores (Błaszkowski 1988 a). The phylogenetic separation, together with the occurrence of the more subtle projections on the spores, when compared to projections on spores of *A. spinosa* and the spiny to tuber-like projections of *A. tuberculata* support *A. spinosissima* as a separate species.

As with several of the AM fungi that were recovered from the West African study area, *A. spinosissima* may similarly be highly sensitive to environmental disturbance and fail to survive the transition from stable forest savanna to intensive agricultural production (Tchabi *et al.* 2008, 2009 a, b; Goto *et al.* 2011), as it was only found in natural forest savannas and in the crop directly cultivated after clearance of this savanna, and not at the other cultivated sites investigated. However, more AM fungal diversity studies in sub-Saharan Africa are needed to describe the biogeographical distribution of *A. spinosissima* and specific AM fungi in general. Quite recently, the fungus was detected by the senior author in the semi-humid tropical Atlantic rainforest biome in Northeastern Brazil indicating that *A. spinosissima* is not restricted to semi-humid tropical Western Africa.

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